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(54) Title: COMPOUNDS WITH GROWTH HORMONE RELEASING PROPERTIES

(57) Abstract

Compounds of the formula A-B-C-D(E)$_p$ are used to stimulate the release of growth hormone from the pituitary.

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COMPOUNDS WITH GROWTH HORMONE RELEASING PROPERTIES

FIELD OF INVENTION

The present invention relates to novel peptide derivatives, compositions containing them, and their use for treating medical disorders resulting from a deficiency in growth hormone.

BACKGROUND OF THE INVENTION

Growth hormone is a hormone which stimulates growth of all tissues capable of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., stimulation of protein synthesis and free fatty acid mobilization and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism. Deficiency in growth hormone can result in a number of severe medical disorders, e.g., dwarfism.

Growth hormone is released from the pituitary. The release is under tight control of a number of hormones and neurotransmitters either directly or indirectly. Growth hormone release can be stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin. In both cases the hormones are released from the hypothalamus but their action is mediated primarily via specific receptors located in the pituitary. Other compounds which stimulate the release of growth hormone from the pituitary have also been described. For example arginine, L-3,4-dihydroxyphenylalanine (L-Dopa), glucagon, vasopressin, PACAP (pituitary adenylyl cyclase activating peptide), muscarinic receptor agonists and a synthethic hexapeptide, GHRP (growth hormone releasing peptide) release endogenous growth hormone either by a direct effect on the pituitary or by affecting the release of GHRH and/or somatostatin from the hypothalamus.

In disorders or conditions where increased levels of growth hormone is desired, the protein nature of growth hormone necessitates parenteral administration. Furthermore, other directly acting natural secretagogues, e.g., GHRH and PACAP, are polypeptides of high molecular weight for which reason parenteral administration is preferred.

The use of shorter peptides for increasing the levels of growth hormone in mammals has previously been proposed, e.g. in EP 18 072, EP 83 864, WO 89/07110, WO 89/01711, WO 89/10933, WO 88/9780, WO 83/02272, WO 91/18016, WO 92/01711 and WO 93/04081.

The structure of growth hormone releasing peptides or peptide derivatives is important for their growth hormone releasing potency as well as their bioavailability. It is therefore the object of the present invention to provide novel peptides with growth hormone releasing properties which have improved properties relative to known peptides of this type.

SUMMARY OF THE INVENTION

A compound of general formula I

$$A\text{-}B\text{-}C\text{-}D(\text{-}E)_p \qquad I$$

wherein p is 0 or 1;

A is hydrogen or $R^1\text{-}(CH_2)_q\text{-}(X)_r\text{-}(CH_2)_s\text{-}CO\text{-}$, wherein

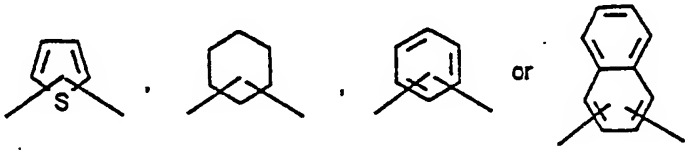
q is 0 or an integer selected from the group: 1, 2, 3, 4, 5;

r is 0 or 1;

s is 0 or an integer selected from the group: 1, 2, 3, 4, 5;

R^1 is hydrogen, imidazolyl, guanidino, piperazino, morpholino, piperidino or $N(R^2)\text{-}R^3$, wherein each of R^2 and R^3 is independently hydrogen or lower alkyl optionally substituted by one or more hydroxyl, pyridinyl or furanyl groups; and

X, when r is 1, is -NH-, $-CH_2-$, -CH=CH-, $-C(R^{16})(R^{17})-$,



wherein each of R^{16} and R^{17} is independently hydrogen or lower alkyl;

B is $(G)_t\text{-}(H)_u$ wherein each of t and u independently is 0 or 1;

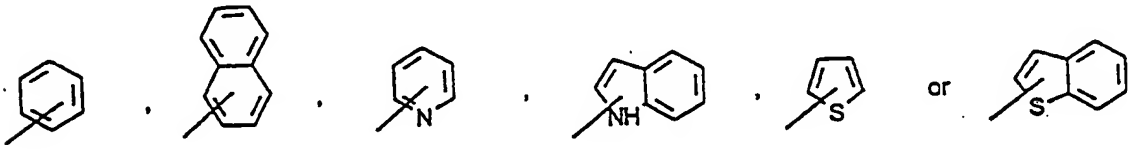
G and H are amino acid residues selected from the group consisting of natural L-amino acids or their corresponding D-isomers, or non-natural amino acids such as 1,4-diaminobutyric acid, amino-isobutyric acid, 1,3-diaminopropionic acid, 4-aminophenylalanine, 3-pyridylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 1,2,3,4-tetrahydronorharman-3-carboxylic acid, N-methylanthranilic acid, anthranilic acid, N-benzylglycine, 3-aminomethylbenzoic acid, 3-amino-3-methyl butanoic acid, sarcosine, nipecotic acid or iso-nipecotic acid;

and wherein, when both t and u are 1, the amide bond between G and H is optionally replaced by Y-NR^{18}-, wherein Y is -CO- or -CH_2-, and R^{18} is hydrogen, lower alkyl or lower aralkyl;

C is a D-amino acid of formula -NH-CH((CH_2)$_w$-R^4)-CO- wherein

w is 0, 1 or 2; and

R^4 is selected from the group consisting of



each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy, lower alkylamino, amino or hydroxy;

D, when p is 1, is a D-amino acid of formula

-NR^{20}-CH((CH_2)$_k$-R^5)-CO-

or, when p is 0, D is -NR^{20}-CH((CH_2)$_l$-R^5)-CH_2-R^6

or -NR^{20}-CH((CH_2)$_m$-R^5)-CO-R^6, wherein

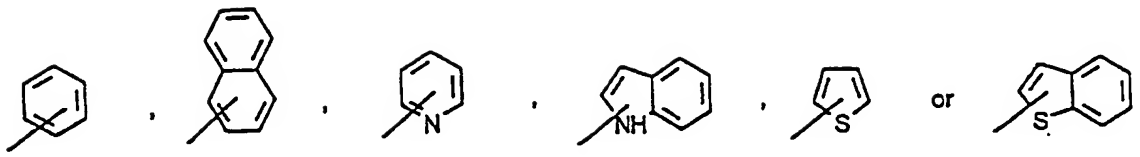
k is 0, 1 or 2;

l is 0, 1 or 2;

m is 0, 1 or 2;

R^{20} is selected from the group consisting of lower alkyl or lower aralkyl;

R^5 is selected from the group consisting of



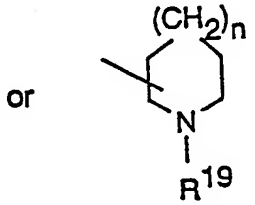
each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy amino or hydroxy; and

R^6 is piperazino, morpholino, piperidino, -OH or -N(R^7)-R^8, wherein each of R^7 and R^8 is independently hydrogen or lower alkyl;

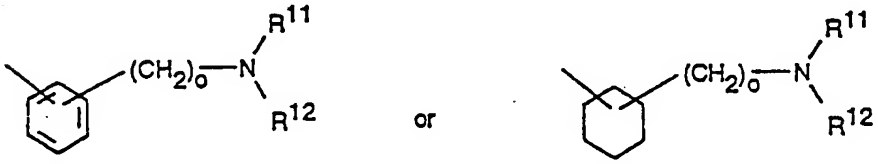
E, when p is 1, is -NH-CH(R^{10})-$(CH_2)_v$-R^9, wherein

v is 0 or an integer selected from the group: 1, 2, 3, 4, 5, 6, 7, 8;

R^9 is hydrogen, imidazolyl, guanidino, piperazino, morpholino, piperidino, -N(R^{11})-R^{12},

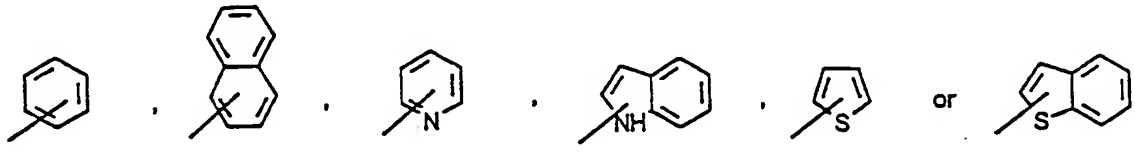


wherein n is 0, 1 or 2, and R^{19} is hydrogen or lower alkyl,



wherein o is an integer selected from the group: 1, 2, 3,

each of R^{11} and R^{12} is independently hydrogen or lower alkyl, or



each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy, amino, alkylamino, hydroxy, or the Amadori rearrangement product from an amino group and a hexapyranose or a hexapyranosyl-hexapyranose

and

R^{10}, when p is 1, is selected from the group consisting of -H, -COOH, $-CH_2-R^{13}$, $-CO-R^{13}$ or $-CH_2-OH$, wherein

R^{13} is piperazino, morpholino, piperidino, -OH or $-N(R^{14})-R^{15}$, wherein each of R^{14} and R^{15} is independently hydrogen or lower alkyl;

all amide bonds within formula I with the exception of the bond between C and D may independently be replaced by $-Y-NR^{18}-$, wherein Y is -CO- or $-CH_2-$, and R^{18} is hydrogen, lower alkyl or lower aralkyl; or a pharmaceutically acceptable salt thereof;

and with the exception of the compounds

(3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-Lys-NH_2

H-Aib-His-D-2Nal-N-Me-D-Phe-Lys-NH_2

H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2

3-(H-Aib-His-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane

2-(H-Aib-His-D-2Nal-N-Me-D-Phe-NH)-2-(1-methyl-2-pyrrolidinyl)ethane

((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2

3-((3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane

2-(H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-pyrrolidinyl)ethane

2-(((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-pyrrolidinyl)ethane

2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-pyrrolidinyl)ethane

3-(H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane

3-(((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane

3-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane

2-((3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-pyrrolidinyl)ethane

2-(((3R)Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-

pyrrolidinyl)ethane.

The peptide derivatives of formula I exhibit an improved resistance to proteolytic degradation by enzymes due to the presence of adjacent D-amino acids in the peptide sequence, optionally combined with the substitution of an amide bond (-CO-NH-) by -Y-NR^{18}- as indicated above, e.g. aminomethylene (-CH_2-NH-) and/or modification at the N- or C-terminal end of the peptide. The increased bioavailability of the peptide derivatives of the invention compared to that of the peptides suggested in the prior art literature is, i. a., thought to be caused by their resistance to proteolytic degradation combined with small size.

In the above structural formulas and throughout the present specification, the following terms have the indicated meanings:

The lower alkyl moities specified above are intended to include those alkyl moities, preferably with 1-6 carbon atoms, of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkyl are methyl, ethyl, propyl, butyl, pentyl, and hexyl. Examples of branched alkyl are isopropyl, sec-butyl, tert-butyl, isopentyl, and isohexyl. Examples of cyclic alkyl are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The lower alkoxy moities specified above are intended to include those alkoxy moities preferably with 1-6 carbon atoms, of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkyloxy are methoxy, ethoxy, propoxy, butoxy, pentoxy, and hexoxy. Examples of branched alkoxy are isopropoxy, sec-butoxy, tert-butoxy, isopentoxy, and isohexoxy. Examples of cyclic alkoxy are cyclopropyloxy, cyclobutyloxy, cyclopentyloxy and cyclohexyloxy.

The lower alkylamino moities specified above are intended to include those alkylamino moities preferably with 1-6 carbon atoms, of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkylamino are methylamino, ethylamino, propylamino, butylamino, pentylamino, and hexylamino. Examples of branched alkylamino are isopropylamino, sec-butylamino, tert-butylamino, isopentylamino, and isohexylamino. Examples of cyclic alkylamino are cyclopropylamino, cyclobutylamino, cyclopentylamino and cyclohexylamino.

In the present context, the term "aryl" is intended to include aromatic rings, such as carbocyclic and heterocyclic aromatic rings selected from the group consisting of phenyl, naphthyl, pyridyl, 1-H-tetrazol-5-yl, thiazolyl, imidazolyl, indolyl, pyrimidinyl, thiadiazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiopheneyl, quinolinyl, pyrazinyl, or isothiazolyl, optionally substituted by one or more C_{1-6}-alkyl, C_{1-6}-alkoxy, halogen, amino or aryl. Aryl is preferably phenyl, thienyl, imidazolyl, pyridyl, indolyl, quinoline or naphthyl optionally substituted with halogen, amino, hydroxy, C_{1-6}-alkyl or C_{1-6}-alkoxy.

The lower aralkyl moities specified above are composed of a lower alkyl moity and a aryl moiety, wherein the lower alkyl moiety and aryl moiety are as defined above.

The term "halogen" is intended to include Cl, F, Br and I.

The common three-letter code is used for natural amino acids, e.g. Ala for alanine.

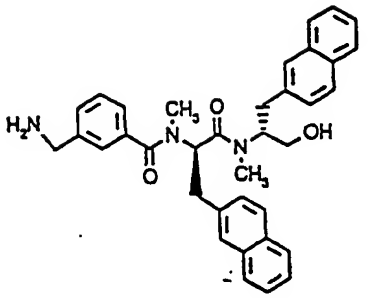
DETAILED DESCRIPTION OF THE INVENTION

In a preferred embodiment of the compound of formula I, A is hydrogen, 3-N-Me-AMB, -3-AMB or Aib. When t is 1, G in the compund of formula I is preferably Ala, Gly, sarcosine, 3-aminomethylbenzoyl, R-nipecotinyl, nipecotic acid or isonipecotic acid, more preferably 3-aminomethylbenzoyl, R-nipecotinyl, nipecotic acid or isonipecotic acid. When u is 1, H is preferably His, Phe, Tic, Phe(4-NH_2), 3-Pyal, Gly, Ala, Sar, Pro, Tyr, Arg, Orn, 3-aminomethylbenzoic acid or D-Phe, more preferably H is His, Phe or Ala, most preferably H is His or Ala. C in the compound of formula I is preferably D-2-naphthylalanine (D-2Nal), D-1-naphthylalanine (D-1Nal), D-Phe or D-Trp, more preferably D-2Nal or D-Phe and most preferably N-Me-D-2Nal, D-2Nal, D-Phe, or N-Me-D-Phe. D in the compound of formula I is preferably -NR^{20}-$CH((CH_2)_k$-$R^5)$-CO-, wherein k is preferably 1 and R^{20} is lower alkyl, more preferably D is D-Phe or D-2Nal. Most preferably D is N-Me-D-Phe-ol, N-Me-D-Phe, N-Me-D-2Nal-ol, N-Me-D-Phe-NH_2, N-Me-D-Phe-NH-Me, or N-Me-D-(4-I)Phe-NH-Me.

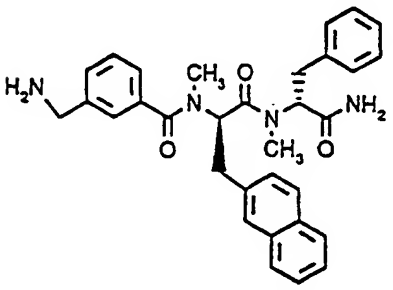
When p is 1in the compound of formula I, E is preferably Lys-NH_2, Ser-NH_2, NH-(2-(1-piperazino)ethyl), NH-(3-(1-morpholino)propyl), NH-(2-aminoethyl), NH-(4-aminomethylbenzyl), NH-(benzyl), Lys-OH, NH-(1-hydroxy-6-amino-2S-hexyl), NH-(2-(1-methyl-2-pyrrolidinyl)ethyl), or 3-N,N-dimethyl-aminopropyl, most preferably E is NH-(2-(1-methyl-2-pyrrolidinyl)ethyl), 3-N,N-dimethyl-aminopropyl, Lys-NH_2, or Ser-NH_2

or R^4 in the compound of formula I is preferably 2-naphthyl. R^5 is preferably phenyl. v is preferably 2-6, and R^9 is NH_2 ,2-morpholinoethyl , 3-morpholinopropyl or (1-methylpyrrolidinyl)ethyl. R^{10} is preferably -COOH, -CH_2-OH, -H, -$CONH_2$ or -$CON(CH_3)_2$.

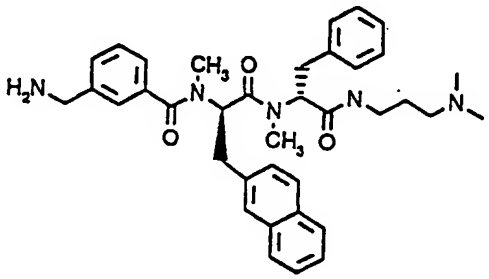
Examples of specific compounds of the present invention are
(2R)-2-((3-Aminomethylbenzoyl))-N-Me-D-2Nal-N-Me)-3-(2-naphthyl)propanol:



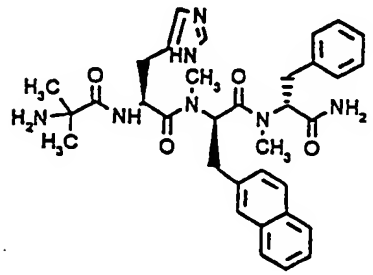
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH_2:



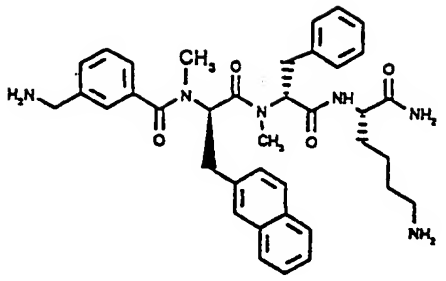
3-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-NMe-D-Phe-NH)-N,N-dimethylaminopropane:



H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH_2:



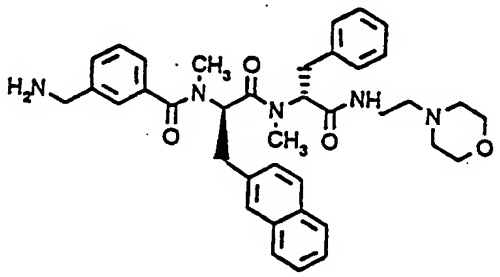
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2:



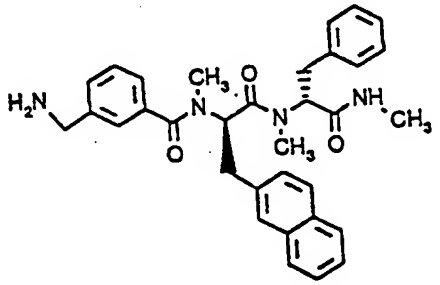
H-Aib-Ala-D-2Nal-N-Me-D-Phe-Lys-NH_2

H-Aib-His-D-2Nal-N-Me-D-Phe-NH_2

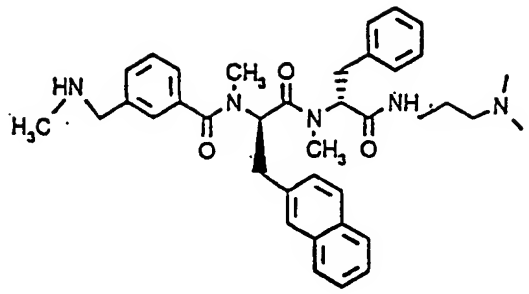
2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinoethane:



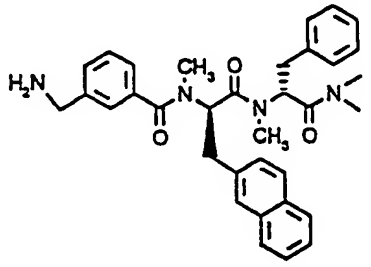
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH-Me:



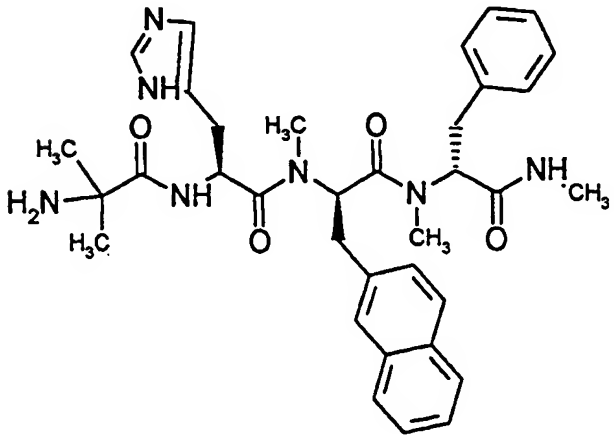
3-((3-Methylaminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-N,N-dimethylaminopropane:



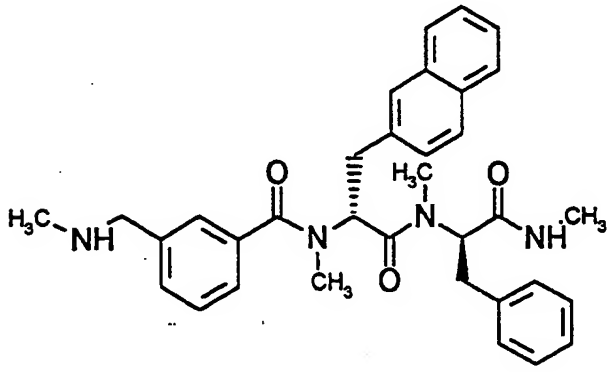
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-N-Me_2:



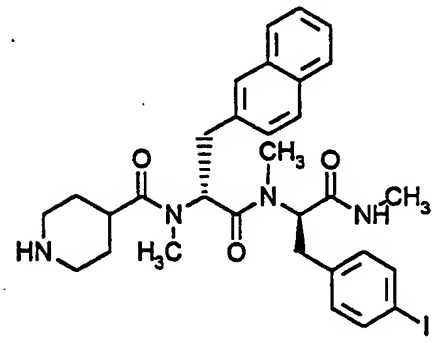
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NHMe:



3-methylaminomethyl-Nme-D-2Nal-Nme-D-Phe-NH-CH_3

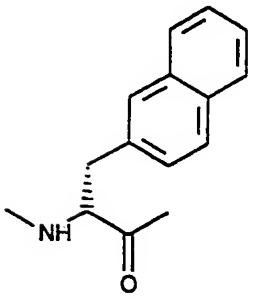


Piperidine-4-carboxylic acid-N-((1R)-1-(N-((1R)-2-(4-iodophenyl)1-(methylcarbamoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylamide

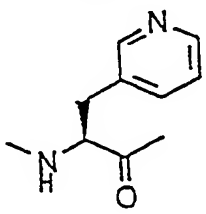


Structures of non-natural amino acid residues:

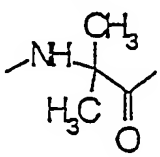
D 2Nal



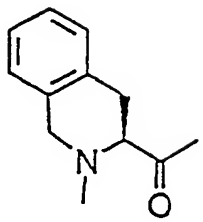
3Pyal



Aib

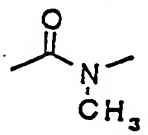


Tic



Abbreviation used for peptide bond substitution:

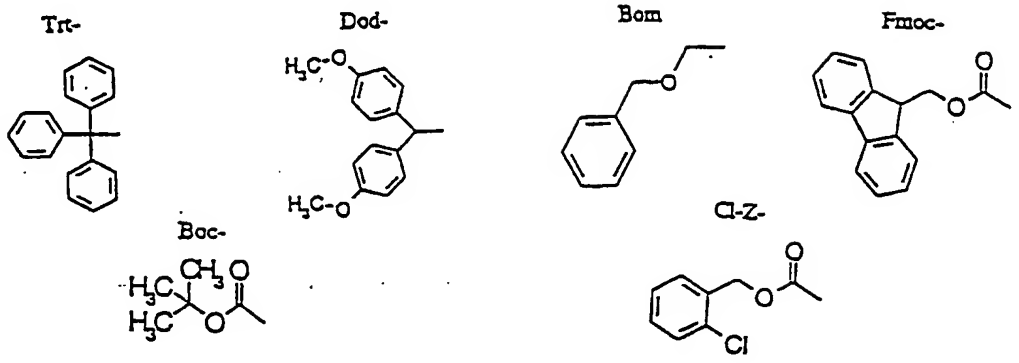
-N-Me-



Compounds of formula I may be prepared by conventional methods of solution or solid phase peptide synthesis. For instance, solid phase synthesis may be carried out substantially as described by Stewart and Young, Solid Phase Peptide Synthesis, 2nd. Ed., Rockford, Illinois, USA, 1976. Solution peptide synthesis may for instance be carried out substantially as described by Bodansky et al., Peptide Synthesis, 2nd. Ed., New York, New York, USA, 1976.

Aminomethylene as a substitution of an amide bond may be introduced according to the method described by Y. Sasaki and D.H. Coy, Peptides 8(1), 1987, pp. 119-121. Peptide derivatives containing a mono- or di-hexapyranose derivatised amino group may be prepared by an Amadori rearrangement substantially by the method described by R. Albert et al., Life Sciences 53, 1993, pp. 517-525. Examples of suitable mono- or di-hexapyranoses are glucose, galactose, maltose, lactose or cellobiose. Derivatives used as starting materials in the synthesis may either be obtained commercially and, when required, provided with suitable protecting groups, or starting materials used to prepare the "A" moiety in general formula I may be prepared by well-known methods and optionally protected in a manner known *per se*.

Abbreviations used for protecting groups:



Pharmaceutically acceptable acid addition salts of compounds of formula I include those prepared by reacting the peptide with an inorganic or organic acid such as hydrochloric, hydrobromic, sulfuric, acetic, phosphoric, lactic, maleic, phthalic, citric, glutaric, gluconic, methanesulfonic, salicylic, succinic, tartaric, oxalic, toluenesulfonic, trifluoracetic, sulfamic and fumaric acid.

In another aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

Pharmaceutical compositions containing a compound of the present invention may be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, 1985. The compositions may appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions, patches or topical applications.

The pharmaceutical carrier or diluent employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water.

Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

If a solid carrier is used for oral administration, the preparation may be tabletted, placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g.

A typical tablet which may be prepared by conventional tabletting techniques may contain:

Core:	
Active compound (as free compound or salt thereof)	100 mg
Colloidal silicon dioxide (Aerosil)	1.5 mg
Cellulose, microcryst. (Avicel)	70 mg
Modified cellulose gum (Ac-Di-Sol)	7.5 mg
Magnesium stearate	
Coating:	
HPMC approx.	9 mg
*Mywacett 9-40 T approx.	0.9 mg

*Acylated monoglyceride used as plasticizer for film coating.

If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

For nasal or pulmonary administration, the preparation may contain a compound of formula I dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g. propylene glycol, surfactants such as bile acid salts polyethylene glycols, polypropylene glycols or polyoxyethylene higher alcohol ethers, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabenes.

For transdermal administration the preparation may be in a form suitable for patches or iontophoresis.

Generally, the compounds of the present invention are dispensed in unit dosage form comprising 0.0001-100 mg of active ingredient together with a pharmaceutically acceptable carrier per unit dosage.

The dosage of the compounds according to this invention is suitably 1-500 mg/day, e.g. about 100 mg per dose, when administered to patients, e.g. humans, as a drug.

It has been demonstrated that compounds of the general formula I possess the ability to release endogenous growth hormone *in vivo*. The compounds may therefore be used in the treatment of conditions which require increased plasma growth hormone levels such as in growth hormone deficient humans or in elderly patients or livestock.

Thus, in a particular aspect, the present invention relates to a pharmaceutical composition for stimulating the release of growth hormone from the pituitary, the composition comprising, as an active ingredient, a compound of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

In a further aspect, the present invention relates to a method of stimulating the release of growth hormone from the pituitary, the method comprising administering to a subject in need thereof an effective amount of a compound of the general formula I or a pharmaceutically acceptable salt thereof.

In a still further aspect, the present invention relates to the use of a compound of the general formula I or a pharmaceutically acceptable salt thereof for the preparation of a medicament for stimulating the release of growth hormone from the pituitary.

The compounds of formula I have interesting pharmacological properties. Examples of such properties are the stimulation of release of growth hormone from the pituitary which has similar effects or uses as growth hormone itself. The uses of

growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of catabolic side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating bone fracture repair, treatment of growth retardation, treating renal failure or insufficiency resulting from growth retardation, treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness, treatment of obesity and growth retardation associated with obesity, treating growth retardation associated with the Prader-Willi syndrome and Turner's syndrome; accelerating the recovery and reducing hospitalization of burn patients; treatment of intrauterine growth retardation, skeletal dysplasia, hypercortisolism and Cushing's syndrome; induction of pulsatile growth hormone release; replacement of growth hormone in stressed patients, treatment of osteochondrodysplasias, Noonan's syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of protein catabolic responses after major surgery, reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and cartilage growth, stimulation of the immune system in companion animals and treatment of disorder of aging in companion animals, growth promoter in livestock and stimulation of wool growth in sheep.

For the above indications the dosage may vary depending on the compound of formula I employed, on the mode of administration and on the therapy desired. However, generally dosage levels between 0.0001 and 100 mg/kg body weight per day may be administered to patients and animals to obtain effective release of endogenous growth hormone. Usually, dosage forms suitable for oral or nasal administration comprise from about 0.0001 mg to about 100 mg, preferably from about 0.001 mg to about 50 mg of the compounds of formula I admixed with a pharmaceutically acceptable carrier or diluent.

The compounds of formula I may be administered in pharmaceutically acceptable acid addition salt form or, where appropriate, as a alkali metal or alkaline earth metal or lower alkylammonium salt. Such salt forms are believed to exhibit approximately the same order of activity as the free base forms.

Optionally, the pharmaceutical composition of the invention may comprise a compound of formula I combined with one or more compounds exhibiting a different activity, e.g., an antibiotic or other pharmacologically active material. This might be another secretagogue, such as GHRP (1 or 6) or GHRH or an analogue thereof, growth hormone or an analogue thereof or a somatomedin such as IGF-1 or IGF-2.

The route of administration may be any route which effectively transports the active compound to the appropriate or desired site of action, such as oral, nasal, pulmonary, transdermal or parenteral, the oral route being preferred.

Apart from the pharmaceutical use of the compounds of formula I, they may be useful in vitro tools for investigating the regulation of growth hormone release.

The compounds of formula I may also be useful in vivo tools for evaluating the growth hormone releasing capability of the pituitary. For example, serum samples taken before and after administration of these compounds to humans can be assayed for growth hormone. Comparison of the growth hormone in each serum sample would directly determine the ability of the patients pituitary to release growth hormone.

Compounds of formula I may be administered to commercially important animals to increase their rate and extent of growth, and to increase milk production.

Pharmacological Methods

Compounds of formula I may be evaluated in vitro for their efficacy and potency to release growth hormone in primary rat somatotrophs.

Rat primary somatotrophs may be prepared essentially as described previously (Chen et al., Endocrinology 1991, 129, 3337-3342 and Chen et al., Endocrinology

1989, 124, 2791-2798). Briefly, rats are killed by decapitation. The pituitary is quickly removed. The pituitaries are digested with 0.2 % collagenase n 0.2 % hyalurinidase in Hanks balanced salt solution. The cells are resuspended in Dulbecco's Modified Eagle's medium containing 0.37 % NaHCO3, 10 % horse serum, 2.5 % fetal calf serum, 1 % nonessential amino acids, 1 % glutamine and 1 % penicillin/streptomycin and adjusted to 1.5 x 105 cells/ml. One ml of this suspension is placed in each well of 24-well trays and left for 2-3 days before release experiments are performed.

On day one of the experiments, cells are washed twice with the above medium containing 25 mM HEPES, pH 7.4. Growth hormone release initiated by addition of medium containing 25 mM HEPES and test compound. Incubation is carried out for 15 minutes at 37°C. After incubation growth hormone released to the medium is measured by a standard RIA.

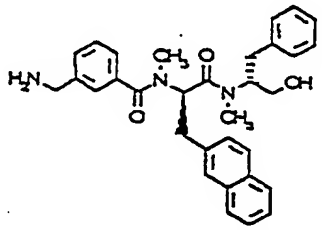
Compounds of formula I may be evaluated for their in vivo effects on growth hormone release in pentobarbital anaesthetized female rats as described previously (Bercu et al. Endocrinology 1991, 129, 2592-2598). Briefly, adult male Sprague-Dawley rats are anesthetized with pentobarbital 50 mg/kg ip. After the rats had been fully anaesthesized the rats are implanted with a tracheal cannula and catheters in the carotid artery and the jugular vein. After a 15 minute recovery, a blood sample is taken at time 0. The pituitary secretagogues are administered iv and artery blood samples are put on ice for 15 minutes and then centrifuged for 2 minutes at 12,000 xg. The serum is decanted and amount of growth hormone determined using a standard RIA.

The invention is further illustrated in the following example which is not in any way intended to limit the scope of the invention as claimed.

The compound prepared in the following example was isolated as the trifluoroacetic acid (TFA) salt.

EXAMPLE 1

Preparation of 2(R)-2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me)-3-phenylpropanol.



165.7 mg of Boc-N-Me-D-2Nal-OH and 165.2 mg of (R)-methylamino-3-phenyl-propan-1-ol (prepared from H-N-Me-D-Phe-OH according to McKennon, M. J.; Meyers, A. I. *J. Org. Chem.* **1993**, 58, 3568-71) and 68.1 mg of HOAt was dissolved in a mixture of 2 ml of DMF and 4 ml of DCM at 0 °C. 115 mg EDAC was added and the mixture was stirred 1h at 0 °C and then 18h at r.t.

The DCM was then removed from the mixture by a stream of nitrogen before 50 ml of EtOAc was added and the resulting mixture was extracted sequentially with 100 ml of 5% aqueous $NaHCO_3$, 100 ml H_2O, 100 ml 5% aqueous $KHSO_4$ and 100 ml H_2O. The resulting organic phase was dried with Na_2SO_4 and concentrated in vacuum on a rotary evaporator to an oil.

502.6 mg 3-Boc-aminomethylbenzoic acid was dissolved in 10 ml DCM by addition of 2 drops of DMF and then converted to the symmetrical anhydride by stirring with 191.6 mg EDAC for 10 min.

A solution of the above lyophilized 2(R)-(H-N-Me-D2Nal-N-Me)-3-phenylpropanol and 342 µl DIEA in 5 ml DCM was added to this mixture and then reacted for 20 h at r.t. The reaction mixture was then concentrated to an oil and redissolved in 50 ml EtOAc. This solution was extracted sequentially with 100 ml 5% aqueous $NaHCO_3$, 100 ml H_2O, 100 ml 5% aqueous $KHSO_4$ and 100 ml H_2O. The resulting organic phase was dried with Na_2SO_4 and concentrated in vacuum on a rotary evaporator to an oil. The oil was then dissolved in 4 ml DCM / TFA 1:1 and stirred. After 10 min the mixture was concentrated by a stream of nitrogen and the resulting oil was redissolved in 20 ml 70% CH_3CN / 0.03 M HCl and 480ml H_2O was added.

The crude product was then purified by semipreparative HPLC in seven runs on a 25 mm x 250 mm column packed with 7µ C-18 silica which was preequilibrated with 28% CH_3CN in 0.05M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with 4M H_2SO_4.

The column was eluted with a gradient of 28% - 38% CH_3CN in 0.05M $(NH_4)_2SO_4$, pH 2.5 at 10 ml/min during 47 min at 40 °C and the peptide containing fractions were collected, diluted with 3 volumes of H_2O and applied to a Sep-Pak® C18 cartridge (Waters part. #:51910) which was equilibrated with 0.1% TFA . The peptide was eluted from the Sep-Pak® cartridge with 70% CH_3CN 0.1% TFA and isolated from the eluate by lyophilisation after dilution with water.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). Mass spectrometry agreed with the expected structure within the experimental error of the method (mass spectrometry ± 0.9 amu).

The RP-HPLC analysis was performed using UV detection at 214 nm and a Vydac 218TP54 4.6mm x 250mm 5µ C-18 silica column (The Separations Group, Hesperia) which was eluted at 1 ml/min at 42 °C. Two different elution conditions were used:

A1: The column was equilibrated with 5% CH_3CN in a buffer consisting of 0.1M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with 4M H_2SO_4 and eluted by a gradient of 5% to 60% CH_3CN in the same buffer during 50 min.

B1: The column was equilibrated with 5% CH_3CN / 0.1% TFA / H_2O and eluted by a gradient of 5% CH_3CN / 0.1% TFA / H_2O to 60% CH_3CN / 0.1% TFA / H_2O during 50 min.

The retention time using elution conditions A1 and B1 was found to be 29.90 min and 31.52 min, respectively.

Synthesis of 3-Boc-aminomethylbenzoic acid

25 g 3-cyanobenzoic acid was dissolved in 70 ml 25% NH_3/H_2O and 200 ml H_2O and 5g 10% Pd/C was added under nitrogen . The mixture was hydrogenated at atmospheric pressure at r.t. while pH was continously adjusted to 10.5 by addition of 12% NH_3/H_2O. After absorption of approximately 4l H_2, during 18 h, the reaction was stopped and the catalyst removed by filtration. The filtrate was concentrated in vac. to 20 ml and unreacted starting material was removed by extraction with ethyl acetate after acidification with 200 ml 1.5 M hydrochloric acid. The aqueous phase was concentrated to dryness and redissolved in 400 ml THF and 343 ml 1 M NaOH. A solution of 30g Boc-anhydride in 100 ml THF was added and the mixture was stirred overnight. Then the reaction mixture was acidified to pH 3 with 1 N HCl

and extracted with 3 x 300 ml of EtOAc. The organic phase was evaporated to a foam. The yield was 22 g.

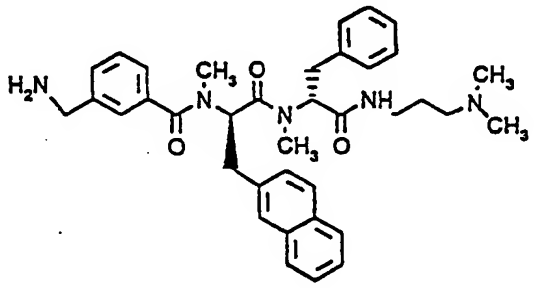
Abbreviations:

r.t. room temperature

EDAC:	N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride
EtOAc:	ethyl acetate
Boc :	t-butyloxycarbonyl
N-Me-D-2Nal :	N-methyl-D-2-naphtylalanine
DCM :	dichloromethane
DIEA :	diisopropylethyl amine
DMF :	N,N-dimethylformamide
HOAt :	1-hydroxy-7-azabenzotriazole
N-Me-D-Phe-ol :	N-methyl-D-phenylalaninol
TFA :	trifluoroacetic acid
THF:	tetrahydrofuran

EXAMPLE 2

3-((3-Aminomethylbenzoyl))-N-Me-D-2Nal-N-Me-D-Phe-NH)-N,N-dimethylaminopropane



Boc-N-Me-D-Phe-OH (279 mg) was dissolved in DMF (4 ml) and stirred 10 min with HOBt (168 mg) and EDAC (230 mg). 3-Dimethylamino-1-propylamine (188 µl) was added and the mixture was stirred 18h at r.t. Then 5% aqueous sodium hydrogen carbonate (50 ml) was added and the resulting mixture was extracted

with EtOAc (50 ml) and the organic phase was dried over Na_2SO_4 and concentrated in vacuum to an oil.

This oil was stirred 10 min at r.t. with TFA/DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in a mixture of 70% CH_3CN (10 ml), 1 N HCl (3 ml) and water (37 ml) and the resulting mixture was immediately frozen and lyophilized.

The product from the lyophilisation was dissolved in DMF (6 ml) and DCM (12 ml). To this mixture was added during stirring Boc-N-Me-D-2Nal-OH (494 mg), HOAt (204 mg), DIEA (171 µl) and after cooling to 0 °C EDAC (288 mg). After stirring for 18h at r.t. the DCM was evaporated by a stream of nitrogen and EtOAc (100 ml) was added. This mixture was extracted two times with 5% aqueous sodium hydrogen carbonate (100 ml) and with water (100 ml) and dried over Na_2SO_4 and concentrated in vacuum to an oil (480 mg).

This oil was stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml). 1 N HCl (1 ml) and water (47 ml) was added and the resulting mixture was immediately frozen and lyophilized to an oil (2 HCl, H-N-Me-D-2Nal-N-Me-D-Phe-NH-$(CH_2)_3$-$N(CH_3)_2$).

Half of the above oil (2 HCl, H-N-Me-D-2Nal-N-Me-D-Phe-NH-$(CH_2)_3$-$N(CH_3)_2$) was dissolved in DCM (9 ml) and 2 drops of DMF and DIEA (342 µl) were added.

This solution was added to a solution of Boc-3AMB-OH (503 mg) and EDAC (192 mg) in DCM (5 ml) which had been stirred for 15 min at r.t.

After stirring for 20 h the reaction mixture was concentrated to an oil with a stream of nitrogen and stirred for 15 min with 5% aqueous sodium hydrogen carbonate (100 ml).

Then EtOAc (50 ml) was added, the organic phase separated and extracted with 5% aqueous sodium hydrogen carbonate (100 ml) and with water (100 ml) and then dried over Na_2SO_4 and concentrated in vacuum to an oil (340 mg).

This oil was stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml) and diluted with water to a final volume of 50 ml.

This crude product was then purified by semipreparative HPLC in eight runs and lyophilized using similar procedures as described in example 1.

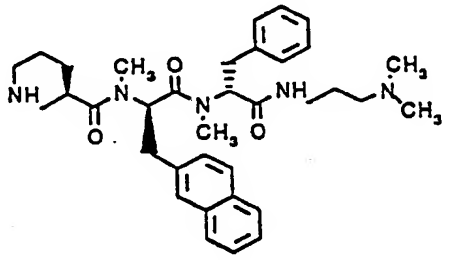
The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:608.2 amu) agreed with the expected structure (teor.

MH^+ 608.8 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 25.23 min and 26.58 min, respectively.

EXAMPLE 3

3-(((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-N,N-dimethylaminopropane



Half of the 2 HCl, H-N-Me-D-2Nal-N-Me-D-Phe-NH-$(CH_2)_3$-$N(CH_3)_2$ which was obtained as an oil in example 2, was dissolved in DCM (9 ml) and 2 drops of DMF and DIEA (342 µl) were added.

This solution was added to a solution of Boc-(R)-nipecotic acid (459 mg) and EDAC (192 mg) in DCM (5 ml) which had been stirred for 15 min at r.t.

After stirring for 20 h the reaction mixture was concentrated to an oil with a stream of nitrogen and stirred for 15 min with 5% aqueous sodium hydrogen carbonate (100 ml)

Then EtOAc (50 ml) was added, the organic phase separated and extracted with 5% aqueous sodium hydrogen carbonate (100 ml) and with water (100 ml) and then dried over Na_2SO_4 and concentrated in vacuum to an oil.

This oil was stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml) and diluted with water to a final volume of 50 ml.

This crude product was then purified by semipreparative HPLC in five runs and lyophilized using similar procedures as described in example 1.

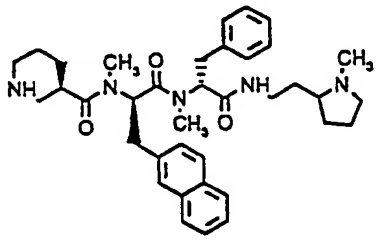
The final product obtained was characterised by analytical RP-HPLC (retention

time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+: 586.3 amu) agreed with the expected structure (teor. MH^+: 585.8 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 25.33 min and 26.35 min, respectively.

EXAMPLE 4

2-(((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-(1-methyl-2-pyrrolidinyl)ethane



Boc-N-Me-D-Phe-OH (279 mg) was dissolved in DMF (10 ml) and stirred 10 min with HOBt (168 mg) and EDAC (384 mg). 2-(Aminoethyl)-1-methyl-pyrrolidine (290 µl) and DIEA (171 µl) were added and the mixture was stirred for 20 h at r.t.

Then the mixture was concentrated to an oil which was dissolved in 50 ml water and lyophilized. The product was redissolved in 25 ml water and then applied to a Sep-Pak® C18 cartridge (Waters part. #:43345) which was equilibrated with 0.03 N hydrochloric acid . The product was eluted from the Sep-Pak® cartridge with 70% CH_3CN in 0.03 N hydrochloric acid and isolated from the eluate by lyophilisation after dilution with water. The resulting material is stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml) and 1 N hydrochloric acid (2 ml) was added. The product was isolated by lyophilisation after dilution with water (50 ml).

The resulting material was dissolved in DMF (3 ml) and stirred 18h at r.t. after addition of Boc-N-Me-D-2Nal-OH (329 mg), HOAt (136 mg), EDAC (230 mg) and DIEA (171µl). Then EtOAc (50 ml) was added and this mixture was extracted with

5% aqueous sodium hydrogen carbonate (50 ml), with 5% aqueous potassium hydrogen sulphate (50 ml) and with water (50 ml). The organic phase was dried with sodium sulphate and concentrated in vac. to an oil.

This oil was stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml) and 1 N hydrochloric acid (3 ml) was added. The product was isolated by lyophilisation after dilution with water (50 ml).

286 mg of this lyophilized product was dissolved in DCM (15 ml) and DIEA (171µl). This solution was added to a solution of Boc-(R)-nipecotic acid (459 mg) and EDAC (192 mg) in DCM (10 ml) which had been stirred for 25 min at r.t.

After stirring for 20 h the reaction mixture was concentrated to an oil by a stream of nitrogen and then redissolved in EtOAc (100 ml) and extracted with 5% aqueous sodium hydrogen carbonate (50 ml), with 5% aqueous potassium hydrogen sulphate (50 ml) and with water (50 ml). The organic phase was dried with sodium sulphate and concentrated in vac. to an oil.

This oil was stirred 10 min at r.t. with TFA / DCM 1:1 (6 ml). After this the TFA / DCM was evaporated by a stream of nitrogen and the resulting oil was dissolved in 70% CH_3CN (10 ml) and diluted with water to a final volume of 50 ml.

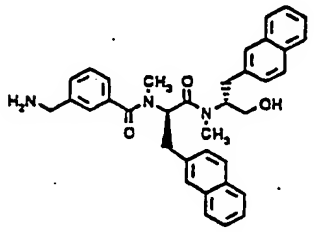
This crude product was then purified by semipreparative HPLC in three runs and lyophilized using similar procedures as described in example 1.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+: 612.2 amu) agreed with the expected structure (teor. MH^+: 612.39 amu) within the experimental error of the method.

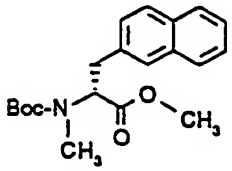
The RP-HPLC retention time using elution condition A1 as defined in example 1. was found to be 25.80 min.

EXAMPLE 5

(2R)-2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me)-3-(2-naphthyl)propanol



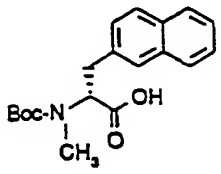
(R)-2-(N-tert-Butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid methyl ester.



(R)-2-tert-Butoxycarbonylamino-3-(2-naphthyl) propionic acid (5,0 g ;16,4 mmol) was dissolved in dry DMF (50 ml). Iodomethane (6,2 ml; 98,4 mmol) and silver(I)oxide (13,3 g ;57,4 mmol) were added and the mixture was stirred overnight. The reaction mixture was filtered and the filtrate was extracted with methylene chloride (200 ml). The organic phase was washed with potassium cyanide (2 x 50 ml; 5%) and water (3 x 75 ml). The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo. The residue was chromatographed (Silica, 5 x 40cm) using ethyl acetate and heptane (1:2) as eluent to afford 4,98 g of (R) 2-(N-tert-butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid methyl ester.

^{1}H-NMR ($CDCl_3$): 1.30, 1.35 (two s, 9H); 2.71, 2.75 (two s, 3H); 3.19, 3.47 (two m, 2H); 3.74, 3.77 (two s, 3H); 4.65, 5.05 (two dd, 1H); 7.29-7.82 (m, 7H) (mixture of rotamers)

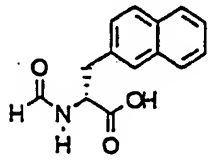
(R)-2-(N-tert-Butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid.



(R)-2-(N-tert-Butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid methyl ester (21,73 g ;65,57 mmol) was dissolved in 1,4-dioxan (200 ml) and water (20 ml) was added. The reaction mixture was cooled on an icebath and lithium hydroxide (1,73 g; 72,13 mmol) was added. After 15 min water (140 ml) was added and the reaction mixture was then stirred for additional 3 hours at room temperature. Ethyl acetate (400 ml) and water (300 ml) were added and pH was adjusted to 2,5 with sodium hydrogen sulfate 1 M (110 ml). The phases was separated and the aqueous phase was extracted with ethyl acetate (200 ml). The combined organic phases were washed with water (300 ml), dried ($MgSO_4$) and the solvent was removed in vacuo to afford 20,1 g af (R) 2-(N-tert-butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid.

^{1}H-NMR (DMSO) 1.18, 1.21 (two s, 9H); 2.62, 2.66 (two s, 3H); 3.11-3.58 (m, 2H); 4.75, 4.90 (two dd, 1H); 7.48-7.88 (m, 7H); 1.85 (s (br), 1H)(mixture of rotamers)

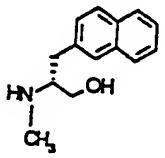
(R) 2-Formylamino-3-(2-naphthyl) propionic acid.



(R)-2-amino-3-(2-naphthyl) propionic acid (18,11 g; 84,14 mmol) was dissolved in formic acid (204 ml) and acetic acid anhydride (70 ml) was added dropwise. The reaction mixture was heated to 55°C and stirred 3½ hours at room temperature. Icecold water (70 ml) was added dropwise and stirred at 0°C for 20 min. The reaction mixture was filtrated and washed with icecold water (20 ml) to afford 20,26 g af (R) 2-formylamino-3-(2-naphthyl) propionic acid.

^{1}H-NMR (DMSO): 3.05 (dd, 1H); 3.27 (dd, 1H); 4.64 (m, 1H); 7.48-7.87 (m, 7H); 7.95 (s, 1H); 8.45 (d, 1H); 12.9 (s (br), 1H).

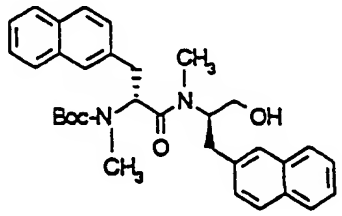
(R)-Methylamino-3-(2-naphthyl) propan-1-ol.



(R)-2-Formylamino-3-(2-naphthyl)propionic acid (4,37 g; 18 mmol) was dissolved in dry tetrahydrofuran (100 ml), and sodium borohydride (1,6 g; 43,2 mmol) was added. Jodine (4,57 g; 18 mmol) was dissolved in dry tetrahydrofuran (40ml) and added dropwise to the reaction mixture at below 40°C. After addition the reaction mixture was heated to reflux for 12 hours. Potassium hydroxide (50 ml; 20%) was added. The aqueous phase was extracted with methyl tertbutyl ether (4 x 50 ml). The combined organic layers were washed with saturated sodium chloride (150 ml), dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was chromatographed (Silica; 5 x 40cm) using DCM/ methanol/ammonia (100:10:1) to afford 1,81 g of (R) methylamino-3-(2-naphthyl)propan-1-ol.

^{1}H-NMR ($CDCl_3$): 2.43 (s, 3H); 2.88-3.05 (m, 3H); 3.10 (s (br), 2H); 3.42 (dd, 1H); 3.69 (dd, 1H); 7.30-7.82 (m, 7H).

N-{(1R)-1-[N-((1R)-2-Hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methylcarbamoyl]-2-(2-naphthyl)ethyl}-N-methylcarbamic acid tert-butyl ester.

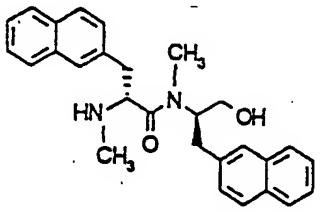


(R)-(N-tert-Butoxycarbonyl-N-methylamino)-3-(2-naphthyl) propionic acid (0,55 g; 1,67 mmol) and (R) methylamino-3-(2-naphthyl) propan-1-ol (0,38 g; 2,00 mmol)

were dissolved in methylene chloride (15 ml) and dimethylformamide (7.5 ml). The reaction mixture was cooled on an icebath. 1-Hydroxy-7-azabenzotriazole (0,24 g; 2,09 mmol) and N-(3-dimethylaminopropyl)-N`-ethylcarbodiimide hydrochloride (0,38 g; 2,0 mmol) were added. The reaction mixture was stirred 12 hours at room temperature. The reaction mixture was concentrated in vacuo. Ethyl acetate (200 ml) was added and the organic solution was washed with water (100 ml), sodium hydrogen carbonate/sodium carbonate (pH 9) (75 ml), sodium hydrogen sulfate (75 ml; 10%) water (100 ml) and dried ($MgSO_4$). The solvent was removed in vacuo and the residue was chromatographed (Silica, 2 x 45cm) using ethyl acetate to afford 0,25g of N-{(1R)-1-(N-[(1R)-2-hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methylcarbamoyl]-2-(2-naphthyl)ethyl}-N-methylcarbamic acid tert-butyl ester.

^{1}H-NMR (DMSO): 0.80-1.99 (several s, 9H); 2.45-4.20 (m, 12 H); 4.70-5.12 (m, 2H) (selected peaks, mixture of rotamers)

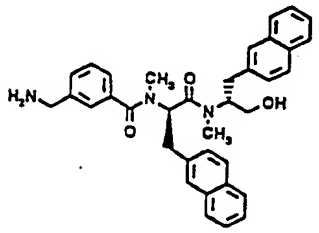
(2R)-N-((1R)-2-hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl) propionamide.



N-{(1R)-1-(N-[(1R)-2-hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methylcarbamoyl]-2-(2-naphthyl)ethyl}-N-methylcarbamic acid tert-butyl ester (0,25 g; 0,475 mmol) was dissolved in DCM (3 ml). Triflouroacetic acid (1 ml) was added and the reaction mixture was stirred for 20 min. The solvent was removed in vacuo. DCM (5 ml) was added and removed in vacuo and repeated. The residue was dissolved in methanol (5 ml). Sodium hydrogen carbonate / sodium carbonate (5 ml; pH 9) was added and the solution was extracted with ethyl acetate (2 x 10ml). The organic phase was dried ($MgSO_4$) and the solvent was removed to afford 0,22 g of (2R)-N-((1R)-2-hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl)propionamide.

^{1}H-NMR ($CDCl_3$): 1.70, 2.37, 2.45, 2.93 (four s, 6H); 2.56-3.05 (m, 2H), 3.52-3.85 (m, 7H); 4.25, 4.97 (two m, 1H); 6.86-7.78 (m, 14 H) (selected peaks, mixture of rotamers)

(2R)-2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me)-3-(2-naphtyl)propanol



3-Boc-aminomethylbenzoic acid (502.6 mg) was dissolved in DCM (6 ml) and then converted to the symmetrical anhydride by stirring with EDAC (191.6 mg) for 15 min.

A solution of (2R)-N-((1R)-2-hydroxy-1-((2-naphthyl)methyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl)propionamide (200 mg) in DCM (5 ml) was added to this mixture and then reacted for 20 h at r.t.

The reaction mixture was then concentrated to an oil and redissolved in EtOAc (100 ml). This solution was extracted sequentially with 5% aqueous $NaHCO_3$ (2 x 50 ml), 5% aqueous $KHSO_4$ (2 x 50 ml) and H_2O (2 x 50 ml). The resulting organic phase was dried with Na_2SO_4 and concentrated in vacuum on a rotary evaporator to an oil. The oil was then dissolved in DCM / TFA 1:1 (6 ml) and stirred. After 10 min the mixture was concentrated by a stream of nitrogen and the resulting oil was redissolved in 70% CH_3CN / 0.1% TFA (5 ml) and diluted with water to a volume of 100 ml.

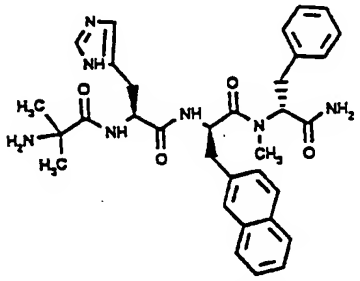
This crude product was then purified by semipreparative HPLC in two runs and lyophilized using similar procedures as described in example 1.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+: 559.5 amu) agreed with the expected structure (teor. MH^+: 560.72 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 33.07 min and 34.63 min, respectively.

EXAMPLE 6

H-Aib-His-D-2Nal-N-Me-D-Phe-NH_2



The peptide was synthesized according to the Fmoc strategy on an Applied Biosystems 431A peptide synthesizer in 0.22 mmol scale using the manufacturer supplied FastMoc UV protocols which employ HBTU mediated couplings in NMP and UV monitoring of the deprotection of the Fmoc protection group. The starting resin used for the synthesis was cat. #: D-1675 from Bachem Feinchemikalien AG, Bubendorf, Switzerland (427) mg which is a Fmoc-2,4-dimethoxy-4'-(carboxymethyloxy)-benzhydryl-amine linked to amino methyl polystyrene resin through an amide bond. The substitution capacity was 0.55 mmol / g . The protected amino acid derivatives used were Fmoc-N-Me-D-Phe-OH, Fmoc-D-2Nal-OH, Fmoc-His(Trt) and Fmoc-Aib-OH. The coupling of Fmoc-N-Me-D-Phe-OH was carried out as a double coupling. After the synthesis the peptide was cleaved from 750 mg of the peptide resin by stirring for 180 min at room temperature with a mixture of 8 ml TFA , 600 mg phenol, 200 µl ethanedithiol, 400 µl thioanisole and 400 µl H_2O. The cleavage mixture was filtered and the filtrate was concentrated to approximately 2 ml by a stream of nitrogen. The crude peptide was precipitated from this oil with 50 ml diethyl ether and washed 2 times with 50 ml diethyl ether.

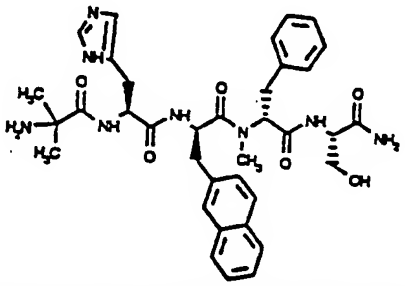
The crude peptide was dried and purified by semipreparative HPLC in one run and lyophilized using similar procedures as described in example 1.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:598.5 amu) agreed with the expected structure (teor. MH^+: 598.73 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 24.68 min and 25.58 min, respectively.

EXAMPLE 7

H-Aib-His-D-2Nal-N-Me-D-Phe-Ser-NH_2

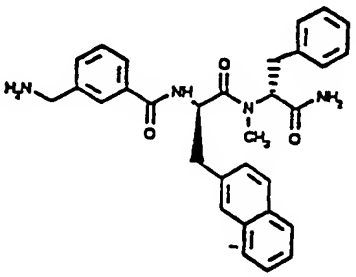


This compound was synthesized using similar procedures as described in example 6. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:685.6 amu) agreed with the expected structure (teor. MH^+: 685.81 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 24.42 min and 25.92 min, respectively.

EXAMPLE 8

(3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-NH_2



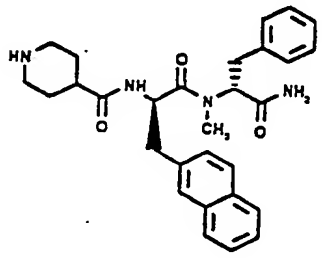
This compound was synthesized using similar procedures as described in example 6. The only exception was that the coupling of Fmoc-D-2Nal-OH was performed using HATU as the activating reagent. H-N-Me-D-Phe-resin (0.23 mmol) was coupled for 150 min with 1 mmol Fmoc-D-2Nal-OH using 1 mmol HATU in the presence of DIEA (2 mmol).

The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:511.2 amu) agreed with the expected structure (teor.

MH^+: 509.6 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 30.73 min and 32.47 min, respectively.

EXAMPLE 9

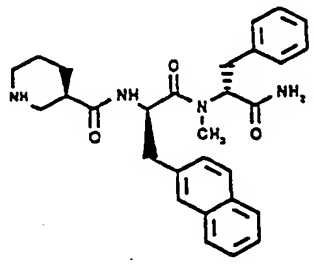
(4-Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH_2



This compound was synthesized using similar procedures as described in example 8. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:486.8 amu) agreed with the expected structure (teor. MH^+: 487.6 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 27.03 min and 28.48 min, respectively.

EXAMPLE 10

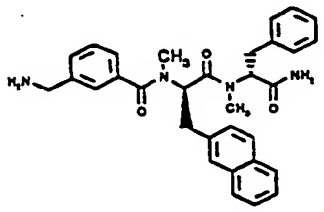
((3R)-3-Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH_2



This compound was synthesized using similar procedures as described in example 8. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:486.9 amu) agreed with the expected structure (teor. MH^+: 487.6 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 28.03 min and 29.50 min, respectively.

EXAMPLE 11

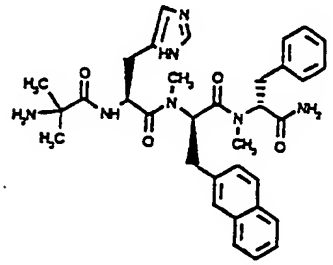
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH_2



This compound was synthesized using similar procedures as described in example 8. with the exceptions that the last residue is introduced by using a symmetrical anhydride coupling. Boc-3-Aminomethylbenzoic acid (251 mg) is stirred for 15 min with EDAC (96 mg) in DCM. Then the resin (429 mg) is added and stirring is continued for 18 h. The other exception is that the time used to cleave the peptide from the resin was reduced to 60 min. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:522.9 amu) agreed with the expected structure (teor. MH^+: 523.6 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 28.83 min and 30.13 min, respectively.

EXAMPLE 12

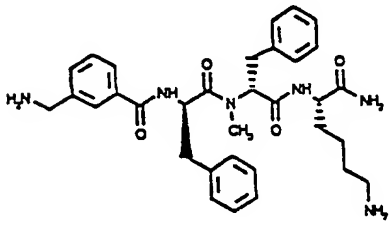
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH_2



This compound was synthesized using similar procedures as described in example 8; where both Fmoc-N-Me-D-2Nal-OH and Fmoc-His(Trt) were coupled using HATU and the time used to cleave the peptide from the resin was reduced to 60 min. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:612.3 amu) agreed with the expected structure (teor. MH^+: 612.8 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1 was found to be 24.33 min and 26.20 min, respectively.

EXAMPLE 13

(3-Aminomethylbenzoyl)-D-Phe-N-Me-D-Phe-Lys-NH_2

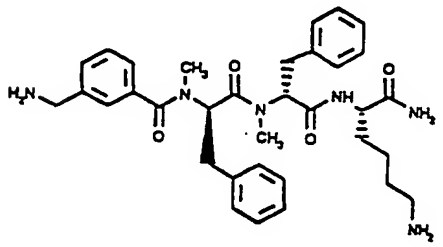


This compound was synthesized using similar procedures as described in example 8. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:587.2 amu) agreed with the expected structure (teor.

MH^+: 586.74 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 21.13 min and 22.60 min, respectively.

EXAMPLE 14

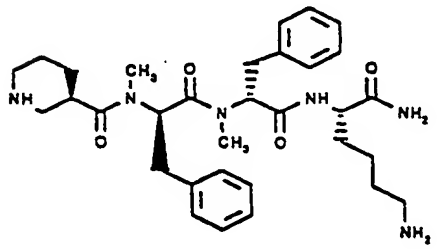
(3-Aminomethylbenzoyl)-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2



This compound was synthesized using similar procedures as described in example 11. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:601.6 amu) agreed with the expected structure (teor. MH^+: 601.77 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 20.40 min and 21.70 min, respectively.

EXAMPLE 15

((3R)-3-Piperidinecarbonyl)-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2

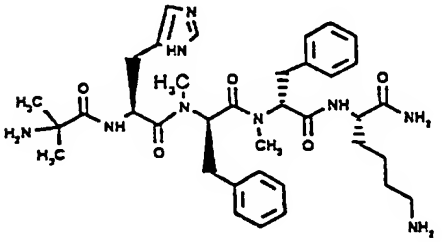


This compound was synthesized using similar procedures as described in example 11. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:579.4 amu) agreed with the expected structure (teor. MH^+: 579.8 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 19.88 min and 21.20 min, respectively.

EXAMPLE 16

H-Aib-His-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2

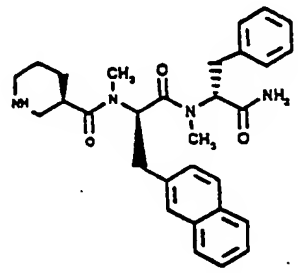


This compound was synthesized using similar procedures as described in example 12. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:690.6 amu) agreed with the expected structure (teor. MH^+: 690.9 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 15.71 min and 17.82 min, respectively.

EXAMPLE 17

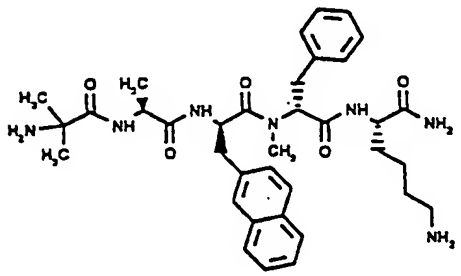
((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH_2



This compound was synthesized using similar procedures as described in example 11. using Fmoc-N-Me-D-Phe-OH, Fmoc-N-Me-D-2Nal-OH and Boc-(R)-Nipecotic acid, where both Fmoc-N-Me-D-2Nal-OH and Boc-(R)-Nipecotic acid were coupled using HATU. The final product obtained was characterised by analytical RP-HPLC (retention time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:500.7 amu) agreed with the expected structure (teor. MH^+: 501.7 amu) within the experimental error of the method.
The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 28.18 min and 29.55 min, respectively.

EXAMPLE 18

H-Aib-Ala-D-2Nal-N-Me-D-Phe-Lys-NH_2



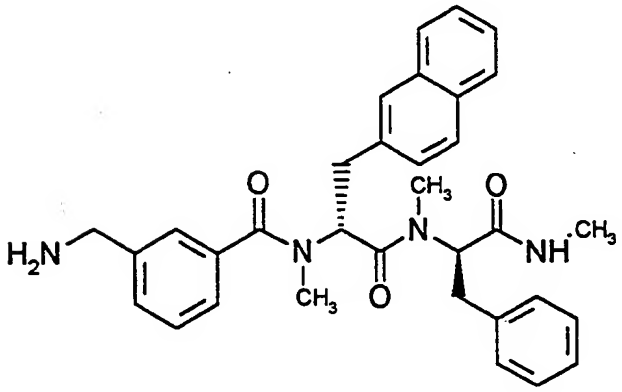
This compound was synthesized using similar procedures as described in example 6. The final product obtained was characterised by analytical RP-HPLC (retention

time) and by Plasma desorption mass spectrometry (molecular mass). The molecular mass found (MH^+:660.7 amu) agreed with the expected structure (teor. MH^+: 660.8 amu) within the experimental error of the method.

The RP-HPLC retention time using elution conditions A1 and B1 as defined in example 1. was found to be 25.63 min and 26.75 min, respectively.

EXAMPLE 19

H-3-Aminomethylbenzoyl-N-Me-D-2Nal-N-Me-D-Phe-NH-CH_3

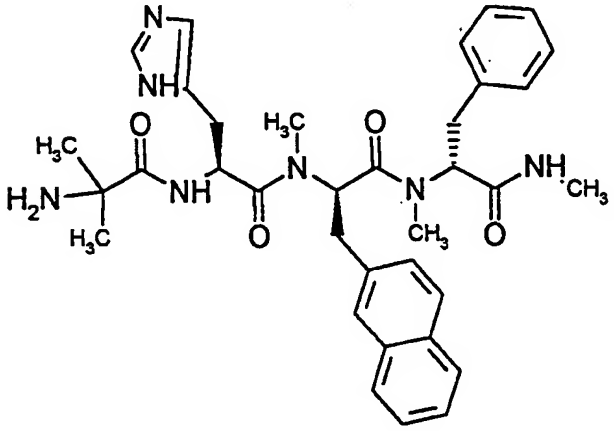


Boc-3AMB-OH (115 mg, 0.458 mmol), 1-hydroxy-7-azabenzotriazole (62 mg, 0.458 mmol) and 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (97 mg, 0.504 mmol) were dissolved in DCM (8 ml) and DMF (1 ml) and stirred for 15 min. N-methyl-2-methylamino-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-3-(2-naphthyl)propionamide (185 mg, 0.458 mmol) dissolved in DCM (5 ml) was added followed by addition of diisopropylethylamine (80 ml, 0.458 mmol) and the mixture was stirred for 20 hours.

The organic phase was washed with sodium hydrogen carbonate (50 ml, 5%) H_2O (50 ml) and saturated NaCl/H_2O (50 ml) and dried with sodium sulfate and evaporated in vacuo. The residue was dissolved in DCM (2 ml) and treated with TFA (2 ml) for 10 min. With a stream of N_2 the volatiles was removed. The residue was dissolved in 50 ml of 20% MeCN and diluted to 500 ml with H_2O.

Semipreparative HPLC
10 ml/min., 5 runs, 30-40% MeCN/0,1 M $(NH_4)_2SO_4$, pH 2,5
Detection 276 nm, Sep-Pals, 70% MeCN/0,1 % TFA, Lyophilization
PD-MS Theory 536,7
Found 535,7± 1
HPLC A1 r_t 31,20 min
B1 r_t 36,35 min

EXAMPLE 20
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NHMe



Fmoc-L-His(Trityl)-OH (1,54 g, 2.48 mmol) (BACHEM B-1570) and 1-hydroxyaza-benzotriazol (338 mg, 2.48 mmol) were dissolved in 9 ml of DMF, cooled to 0-4°C and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (475 mg, 2.48 mmol) was added. The reaction mixture was stirred for 15 min. at 0-4° C.
N-Methyl-2-methylamino-N-((1R)-1-(Methylcarbamoyl)-2-phenylethyl)-3-(2-naphthyl)propionamid (500 mg, 1.24 mmol) dissolved in methylen chloride (18 ml) was cooled to 0-4° C and added and stirred for 1 hour at 0-4° C followed by addition of diisopropylethylamine (0.425 ml, 2.48 mmol). The temperature of the mixture was slowly raised to room temperature and the mixture was stirred for 72 hours. DCM was evaporated in a stream of N_2 and to the mixture was added 100 ml ethyl acetate and washed with sodium hydrogen carbonate (2 x 100 ml, 5%) and potassium hydrogen

sulfate (100 ml, 5%). The phases were separated and the organic phase was dried with sodium sulfate and evaporated in vacuo. The residue was dissolved in DMF (8 ml) and treated with piperidine for 15 min., diluted with H_2O (100 ml) and quenched with acetic acid (1,5 ml). Acetonitrile was added and the mixture was diluted with H_2O to 250 ml. The clear solution was applied to a 10 g "Seppaks" #Water, washed with H_2O/0,03 m HCl and eluted with 50 ml 35% MeCN/0,03 M HCl. Deluted with H_2O to 200 ml and lyophilized.

Boc-α aminoisobutyric acid (756 mg, 3.72 mmol), 1-hydroxyazabenzotriazole hydrate (506 mg, 3.72 mmol) and 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (713 mg, 3.72 mmol) were dissolved in DMF (6 ml) and after 15 min. was added H-L-His(trityl)-NMeD2Nal-NMeDPhe-$NHCH_3$, 2 HCL dissolved in DCM (12 ml) followed by addition of diisopropylethylamine (0.637 ml) and stirred for 72 hours. DCM was evaporated in a stream of N_2 and the mixture was added 100 ml ethyl acetate and washed with sodium hydrogen carbonate (2 x 50 ml, 5%) and potassium hydrogen sulfate (50 ml, 5%). The phase were separated and the organic phase was dried with sodium sulfate and evaporated in vacuo. The residue was dissolved in DCM (6 ml), cooled to 0-4° C and treated with TFA (6 ml) for 10 min. at 0-4° C. With a stream of N_2 the volatiles was removed. The oily residue was dissolved in 35 ml of 70% acetonitrile diluted with H_2O to 50 ml and added 10 ml of concentrated hydrochloric acid (12 molar) and stirred for 72 hours. The mixture was diluted to 200 ml with H_2O and neutralized with solid sodium carbonate, finally diluted to 400 ml with H_2O.

Semipreparative HPLC

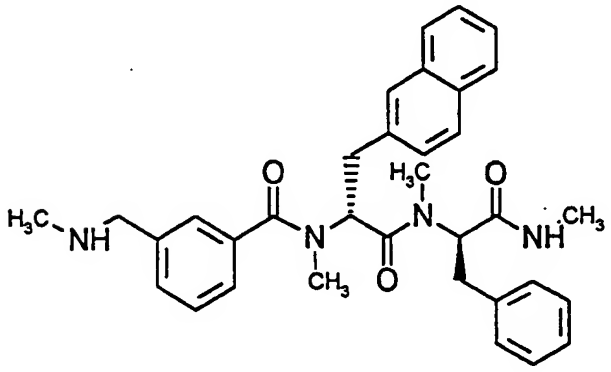
PD-MS, theory: 550,7, found 550,1

HPLC A1 r_t. 31,75 min.

B1 r_t. 36,15 min.

EXAMPLE 21

3-methylaminomethylbenzoyl-N-Me-D-2Nal-N-Me-D-Phe-NH-CH_3



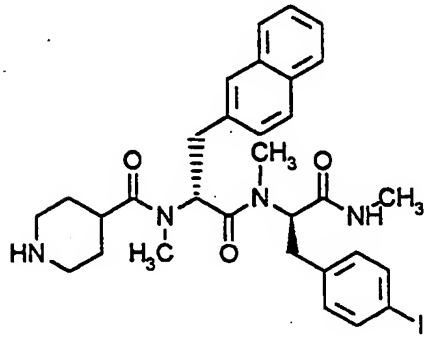
Boc-NMe3AMB-OH (658 mg, 2.48 mmol), 1-hydroxyazabenzotriazole hydrate (338 mg, 2.48 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (475 mg, 2.48 mmol) were dissolved in 6 ml of DMF and stirred for 15 min. N-Methyl-2-methylamino-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)-3-(2-naphthyl)propionamid (500 mg, 1.24 mmol) dissolved in methylene chloride (12 ml) was added, followed by addition of diisopropylethylamine (0,425 ml, 2.48 mmol). The mixture was stirred for 20 hours.

DCM was evaporated in a stream of N_2 and to the mixture was added 75 ml of ethyl acetate and washed with sodium hydrogencarbonate (2 x 50 ml, 5%) and potassium hydrogen sulfate (50 ml, 5%). The phases were separated and the organic phase was dried with sodium sulfate and evaporated in vacuo. The residue was dissolved in 10 ml methylene chloride, cooled to 0-4° C and treated with TFA (10 ml) for 10 min. at 0-4° C. With a stream of N_2 the volatiles were removed. The oily residue was dissolved in 25 ml of 70% MeCN/0,1 % TFA and diluted to 600 ml with H_2O.

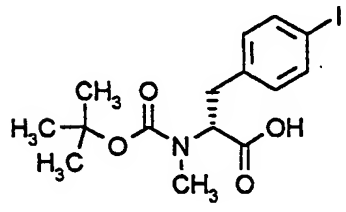
Semipreparative HPLC
Large column, 40 ml/min., 8 runs 28-40& P 11 $(NH_4)_2SO_4$, 276 nM.
Seppak, Lyophilization
PD-MS Theory: 550,7, found 550,1
HPLC A1 r_t 31,75 min
B1 r_t 36,15 min

EXAMPLE 22

Piperidine-4-carboxylic acid N-((1R)-1-(N-((1R)-2-(4-iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylamide



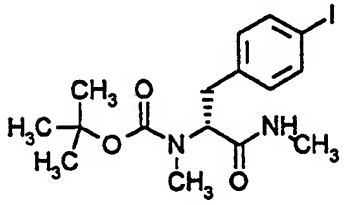
(2R)-2-(N-tert-butoxycarbonyl-N-methylamino)-3-(4-iodophenyl)propionic acid



Prepared according to Can J. Chem. (1977), 55, 906.

^{1}H-NMR: ($CDCl_3$) d 1.34 (s, 4.5H), 1.38 (s, 4.5H), 2.70 (s,1.5H), 2.75 (s, 1.5H); 2.85-3.10 (m, 1H), 3.2-3.4 (m, 1H); 4.4-4.6 (m, 0.5H), 6.9-7.0 (m, 2H), 7.62 (d, J=10 Hz, 2Hz), 9.5-10 (bs, 1H)

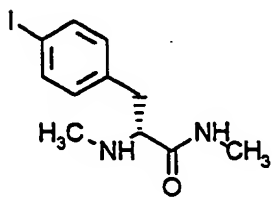
N-((1R)-2-(4-Iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methylcarbamic acid tert-butylester



(2R)-2-(N-tert-butoxycarbonyl-N-methylamino)-3-(4-iodophenyl)propionic acid (2.00 g, 4.9 mmol) was dissolved in methylene chloride (20 ml). Hydroxybenzotriazole hydrate (0.67 g, 4.9 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimid hydrochloride (0.99 g, 4.9 mmol) were added and the mixture was stirred for 15 minutes. Methylamine (0.38 g of a 40% solution in methanol, 4.9 mmol) was added and the mixture was stirred overnight. Methylene chloride (40 ml) was added and the mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate (50 ml) and a solution of sodium hydrogen sulphate (10%, 50 ml). The organic phase was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was chromatographed on silica (2.5 x 20 cm) using ethyl acetate/heptane (2:1) as eluent to afford 1.77 g of N-((1R)-2-(4-iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methylcarbamic acid tert-butylester.

^{1}H-NMR: ($CDCl_3$) (selected peaks for major rotamer) d 1.39 (s, 9H); 2.75 (s, 3H); 2.80 (d, 3H); 3.29 (dd, 1H); 4.88 (t, 1H).

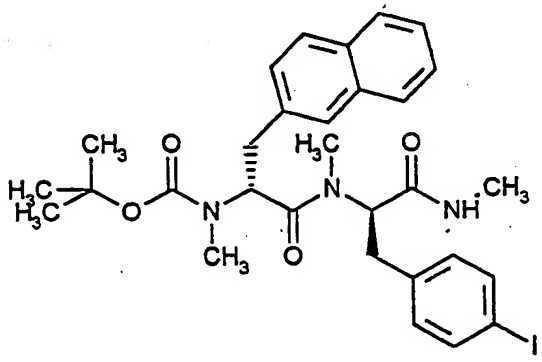
(2R)-3-(4-Iodophenyl)-N-methyl-2-(methylamino)propionamide



N-((1R)-2-(4-Iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methylcarbamic acid tert-butylester (1.7 g; 4.0 mmol) was dissolved in methylene chloride (10 ml) and trifluoroacetic acid (5 ml) was added. The mixture was stirred for 1 h. Methylene chloride (30 ml) and water (30 ml) was added. Solid sodium hydrogen carbonate was added to pH 8. The organic phase was separated, dried ($MgSO_4$) and evaporated in vacuo to afford 1.22 g of (2R)-3-(4-iodophenyl)-N-methyl-2-(methylamino)propionamide.

H-NMR: ($CDCl_3$) d 2.28 (s, 3H); 2.68 (dd, 1H); 2.81 (d, 3H); 3.08-3.19 (m, 2H); 6.95 (d, 2H); 7.63 (d, 2H)

N-Methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-(4-iodophenyl)ethyl)carbamoyl)-2-(2-naphthyl)ethyl)carbamic acid tert-butylester

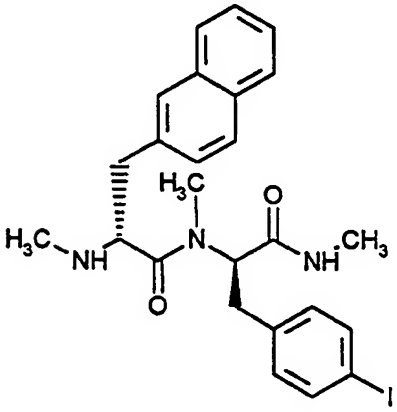


(2R)-2-(N-tert-butoxycarbonyl-N-methylamino)-3-(2-naphthyl)propionic acid (1.10 g; 3.30 mmol) was dissolved in methylene chloride (10 ml) and HOAt (0.45 g, 3.1 mmol) and EDAC (0.66 g, 3.5 mmol) were added. After stirring for 15 min, (2R)-3-(4-iodophenyl)-N-methyl-2-(methylamino)propionamide (1.0 g, 3.1 mmol) and diisopropylethylamine (0.45 g, 3.4 mmole) were added and the mixture was stirred overnight. Methylene chloride (30 ml) was added and the mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate (30 ml) and a solution of sodium hydrogen sulphate (10%, 30 ml). The organic phase was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was chromatographed on silica (2.5 x 20 cm) using ethyl acetate/heptane (2:1) as eluent to afford 1.74 g of N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-(4-

iodophenyl)ethyl)carbamoyl)-2-(2-naphthyl)ethyl)carbamic acid tert-butylester.

^{1}H-NMR: ($CDCl_3$) (selected peaks for major rotamer) d 1.38 (s, 9H); 2.18 (d, 3H); 2.45 (s, 3H); 2.75 (s, 3H)5.05 (m, 1H); 5.42 (m, 1H).

(2R)-N-((1R)-2-(4-Iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl)propionamide

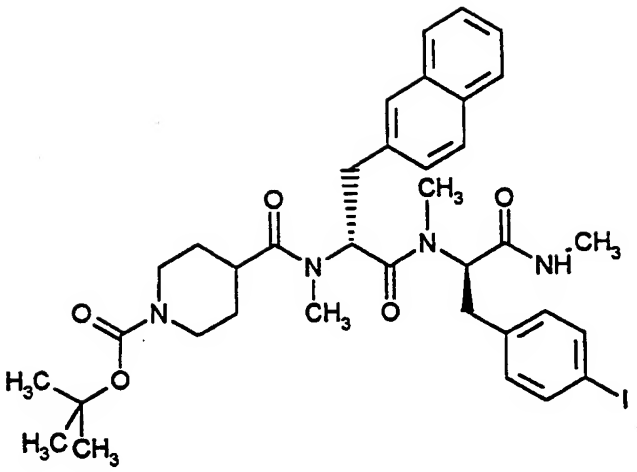


N-Methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-(4-iodophenyl)ethyl)carbamoyl)-2-(2-naphthyl)ethyl)carbamic acid tert-butylester was dissolved in a mixture of methylene chloride and trifluoroacetic acid and stirred for 15 min.

Methylene chloride (20 ml) and water (30 ml) were added. Solid sodium hydrogen carbonate was added to pH 8. The organic phase was separated, dried ($MgSO_4$) and evaporated in vacuo to afford 1.40 g of (2R)-N-((1R)-2-(4-iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl)propionamide.

^{1}H-NMR: ($CDCl_3$) (selected peaks for major rotamer) d 1.79 (s, 3H); 2.02 (d, 3H); 2.55 (s, 3H); 3.78 (dd, 1H); 5.44 (dd, 1H).

4-(N-((1R)-1-(N-((1R)-2-(4-Iodophenyl)-1-(methylcarbomoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylcarbamoyl)piperidine-1-carboxylic acid tert-butylester



1-tert-butoxycarbonylpiperidine-4-carboxylic acid (143 mg, 0.66 mmol) was dissolved in methylene chloride (10 ml) and HOAt (90 mg, 0.66 mmol) and EDAC (140 mg, 0.73 mmol) were added. After 15 min of stirring (2R)-N-((1R)-2-(4-iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methyl-2-methylamino-3-(2-naphthyl)propionamide (350 mg, 0.66 mmol) and diisopropylethylamine (85 mg, 0.66 mmol) were added and the mixture was stirred overnight. Methylene chloride (20 ml) was added and the mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate (20 ml) and a solution of sodium hydrogen sulphate (10%, 20 ml). The organic phase was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was chromatographed on silica (2.5 x 20 cm) using ethyl acetate as eluent to afford 412 mg of
4-(N-((1R)-1-(N-((1R)-2-(4-iodophenyl)-1-(methylcarbomoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylcarbamoyl)piperidine-1-carboxylic acid tert-butylester.

4-(N-((1R)-1-(N-((1R)-2-(4-Iodophenyl)-1-(methylcarbomoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylcarbamoyl)piperidine-1-carboxylic acid tert-butyl ester (412 mg 0.56 mmol) was dissolved in a mixture of methylene chloride (5 ml) and trifluoroacetic acid (5 ml) and stirred for 5 min. Methylene chloride (20 ml) and a saturated aqueous solution of sodium hydrogencarbonate (20 ml) was added. Solid sodium hydrogen carbonate was added to pH 8. The phases were separated and the organic phase was dried

($MgSO_4$) and evaporated to afford 255 mg of the title compound.

^{1}H-NMR: ($CDCl_3$) (selected peaks for major rotamer) d 2.32 (d, 3H); 2.58 (s, 3H); 2.68 (s,3H); 5.33 (m, 1H); 5.84 (t, 1H)

HPLC: r_t = 33.35 min (A1)
PDMS: m/z 640.8 $(M+H)^+$.

Abbreviations

HBTU	O-(Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
NMP	N-methyl pyrrolidone
HATU	O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Trt-	Trityl
HOBT	7-hydroxybenzotriozole hydrate
3-AmB	3-Aminomethylbenzoyl
N-Me-3-AMB	3-methylaminomethylbenzoyl

CLAIMS

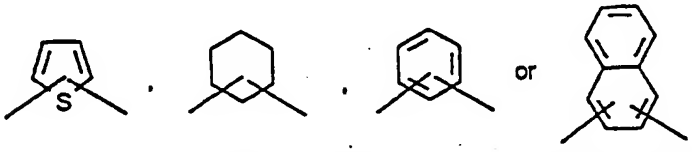
1. A compound of general formula I

$$A\text{-}B\text{-}C\text{-}D(\text{-}E)_p \qquad I$$

wherein p is 0 or 1;

A is hydrogen or $R^1\text{-}(CH_2)_q\text{-}(X)_r\text{-}(CH_2)_s\text{-}CO\text{-}$, wherein
q is 0 or an integer selected from the group: 1, 2, 3, 4, 5;
r is 0 or 1;
s is 0 or an integer selected from the group: 1, 2, 3, 4, 5;
R^1 is hydrogen, imidazolyl, guanidino, piperazino, morpholino, piperidino or $N(R^2)\text{-}R^3$, wherein each of R^2 and R^3 is independently hydrogen or lower alkyl optionally substituted by one or more hydroxyl, pyridinyl or furanyl groups; and

X, when r is 1, is -NH-, $-CH_2-$, -CH=CH-, $-C(R^{16})(R^{17})-$,

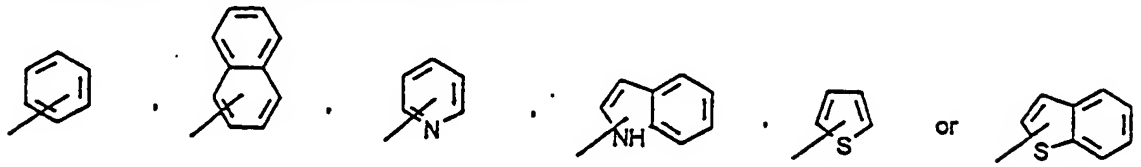


wherein each of R^{16} and R^{17} is independently hydrogen or lower alkyl;
B is $(G)_t\text{-}(H)_u$ wherein each of t and u independently is 0 or 1;
G and H are amino acid residues selected from the group consisting of natural L-amino acids or their corresponding D-isomers, or non-natural amino acids such as 1,4-diaminobutyric acid, amino-isobutyric acid, 1,3-diaminopropionic acid, 4-aminophenylalanine, 3-pyridylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 1,2,3,4-tetrahydronorharman-3-carboxylic acid, N-methylanthranilic acid, anthranilic acid, N-benzylglycine, 3-aminomethylbenzoic acid, 3-amino-3-methyl butanoic acid, sarcosine, nipecotic acid or iso-nipecotic acid;
and wherein, when both t and u are 1, the amide bond between G and H being optionally replaced by $Y\text{-}NR^{18}\text{-}$, wherein Y is -CO- or $-CH_2-$, and R^{18} is hydrogen, lower alkyl or lower aralkyl;

C is a D-amino acid of formula -NH-CH($(CH_2)_w$-R^4)-CO- wherein

w is 0, 1 or 2; and

R^4 is selected from the group consisting of



each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy, lower alkylamino, amino or hydroxy;

D, when p is 1, is a D-amino acid of formula -NR^{20}-CH($(CH_2)_k$-R^5)-CO-

or, when p is 0, D is -NR^{20}-CH($(CH_2)_l$-R^5)-CH_2-R^6 or -NR^{20}-CH($(CH_2)_m$-R^5)-CR

^{6}, wherein

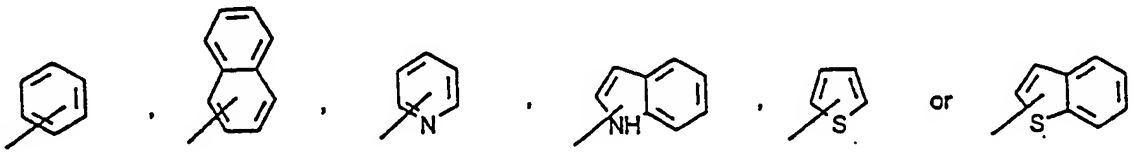
k is 0, 1 or 2;

l is 0, 1 or 2;

m is 0, 1 or 2;

R^{20} is selected from the group consisting of lower alkyl or lower aralkyl;

R^5 is selected from the group consisting of



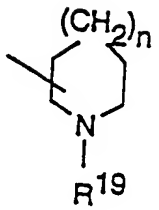
each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy amino or hydroxy; and

R^6 is piperazino, morpholino, piperidino, -OH or -N(R^7)-R^8, wherein each of R^7 and R^8 is independently hydrogen or lower alkyl;

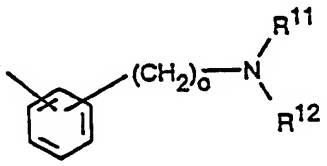
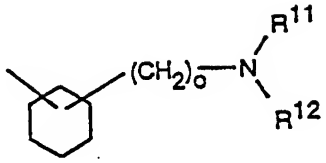
E, when p is 1, is -NH-CH(R^{10})-($(CH_2)_v$-R^9, wherein

v is 0 or an integer selected from the group: 1, 2, 3, 4, 5, 6, 7, 8;

R^9 is hydrogen, imidazolyl, guanidino, piperazino, morpholino, piperidino, -N(R^{11})-R^{12},

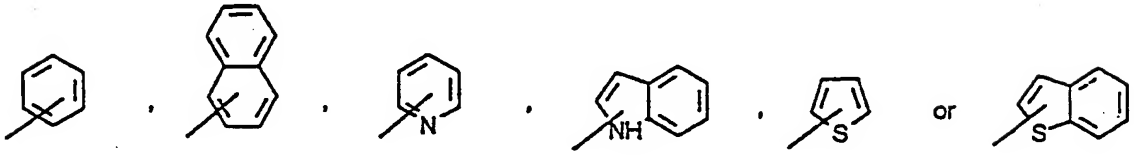
or 

wherein n is 0, 1 or 2, and R^{19} is hydrogen or lower alkyl,

 or 

wherein o is an integer selected from the group: 1, 2, 3,

each of R^{11} and R^{12} is independently hydrogen or lower alkyl, or



each of which is optionally substituted with halogen, lower alkyl, lower alkyloxy, amino, alkylamino, hydroxy, or the Amadori rearrangement product from an amino group and a hexapyranose or a hexapyranosyl-hexapyranose

and

R^{10}, when p is 1, is selected from the group consisting of H, -COOH, $-CH_2-R^{13}$, $-CO-R^{13}$ or $-CH_2-OH$, wherein

R^{13} is piperazino, morpholino, piperidino, -OH or $-N(R^{14})-R^{15}$, wherein each of R^{14} and R^{15} is independently hydrogen or lower alkyl;

all amide bonds within formula I with the exception of the bond between C and D may independently be replaced by $-Y-NR^{18}-$, wherein Y is -CO- or $-CH_2-$, and

R^{18} is hydrogen, lower alkyl or lower aralkyl; or a pharmaceutically acceptable salt thereof; and with the exception of the compounds

(3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-Lys-NH_2,

H-Aib-His-D-2Nal-N-Me-D-Phe-Lys-NH_2,

H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2,

3-(H-Aib-His-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane,
2-(H-Aib-His-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane,
((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2,
3-((3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-NH)1-morpholinopropane,
2-(H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane,
2-(((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane,
2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane,
3-(H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane,
3-(((3R)-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholino-propane,
3-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinopropane,
2-((3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane,
2-(((3R)Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane.

2. A compound according to claim 1, wherein A is hydrogen, 3-AMB, N-Me-3-AMB or Aib

3. A compound according to claim 1, wherein t is 1 and u is 0 and G is selected from the group consisting of 3-aminomethylbenzoyl, nipecotic acid and isonipecotic acid.

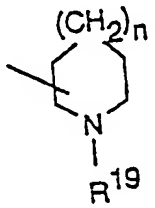
4. A compound according to claim 1, wherein t is 1, u is 1, G is Aib, and H is selected from the group consisting of His, Phe and Ala.

5. A compound according to claim 1, wherein C is selected from the group consisting of D-2-Nal and D-Phe.

6. A compound according to claim 1, wherein, when p is 1, D is D-Phe or D-2Nal.

7. A compound according to claim 1, wherein, when p is 0, D is D-Phe-NH_2 or D-2Nal-NH_2.

8. A compound according to claim 1, wherein E, when p is 1, is -NH-CH(R^{10})-(CH_2)$_v$-R^9, wherein
v is 0 or an integer selected from the group: 1,2,3,4;
R^9 is hydrogen, morpholino, piperidino, N(R^{11})-R^{12}

or 

wherein n is 0, 1 or 2, and R^{19} is hydrogen or lower alkyl,
each of R^{11} and R^{12} is independently hydrogen or lower alkyl, and

R^{10}, when p is 1, is selected from the group consisting of -H, -COOH, -CH_2-R^{13}, -CO-R^{13} or -CH_2-OH, wherein R^{13} is piperazino, morpholino, piperidino, -OH or -N(R^{14})-R^{15}, wherein each of R^{14} and R^{15} is independently hydrogen or lower alkyl.

9. A compound according to claim 1, wherein at least one of the amide bonds between A and B, between B and C, between D and (E)$_p$ and between G and H is replaced by -CO-N(CH_3)-.

10. A compound selected from the group consisting of
(R)-2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me)-3-phenylpropanol, or the TFA salt thereof;
3-((3-Aminomethylbenzoyl))N-Me-D-2Nal-N-Me-D-Phe-NH)-1-1N,N-dimethylaminopropane, or the TFA salt thereof;
3-(((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-N,N-dimethylaminopropane, or the TFA salt thereof;
2-(((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-(1-methyl-2-pyrrolidinyl)ethane, or the TFA salt thereof;
H-Aib-His-D-2Nal-N-Me-D-Phe-Ser-NH_2, or the TFA salt thereof;
(3-Aminomethylbenzoyl)-D-2Nal-N-Me-D-Phe-Lys-NH_2, or the TFA salt thereof;
(4-Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
((3R)-3-Piperidinecarbonyl)-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
(3-Aminomethylbenzoyl)-D-Phe-N-Me-D-Phe-NH_2, or the TFA salt thereof;
(3-Aminomethylbenzoyl)-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2, or the TFA salt thereof;

((3R)-3-Piperidinecarbonyl)-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2, or the TFA salt thereof;
H-Aib-His-N-Me-D-Phe-N-Me-D-Phe-Lys-NH_2, or the TFA salt thereof;
((3R)-3-Piperidinecarbonyl)-N-Me-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
(2R)-2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me)-3-(2-naphthyl)propanol, or the TFA salt thereof;
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
3-((3-Aminomethylbenzoyl)-N-Me-D-Phe-NH)-1-N,N-dimethylaminopropane,
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-Lys-NH_2,
H-Aib-Ala-D-2Nal-N-Me-D-Phe-Lys-NH_2, or the TFA salt thereof;
H-Aib-His-D-2Nal-N-Me-D-Phe-NH_2, or the TFA salt thereof;
2-((3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-morpholinoethane,
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH-Me,
3-((3-Methylaminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-NH)-1-N,N-dimethylaminopropane,
(3-Aminomethylbenzoyl)-N-Me-D-2Nal-N-Me-D-Phe-N-Me_2,
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NH_2,
3-Aminomethylbenzoyl-N-Me-D-2Nal-N-Me-D-Phe-NH-CH_3, or the TFA salt thereof;
3-methylaminomethylbenzoyl-N-Me-D-2Nal-N-Me-D-Phe-NH-CH_3, or the TFA salt thereof;
H-Aib-His-N-Me-D-2Nal-N-Me-D-Phe-NHMe, or the HCl salt thereof;
and Piperidine-4-carboxylic acid-N-((1R)-1-(N-((1R)-2-(4-iodophenyl)-1-(methylcarbamoyl)ethyl)-N-methylcarbamoyl)-2-(2-naphthyl)ethyl)-N-methylamide,

or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising, as an active ingredient, a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

12. A composition according to claim 11 in unit dosage form, comprising from about 10 to about 200 mg of the compound according to any one of claims 1 to 10

or a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition for stimulating the release of growth hormone from the pituitary, the composition comprising, as an active ingredient, a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

14. A pharmaceutical composition according to claim 11, 12, or 13 for oral, transdermal, nasal, pulmonary, or parenteral administration.

15. A method of stimulating the release of growth hormone from the pituitary, the method comprising administering to a subject in need thereof an effective amount of a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof, or of a composition according to any one of the claims 11, 12 or 14.

16. A method according to claim 15, wherein the effective amount of the compound according to any one of claims 1 to 10 or pharmaceutically acceptable salt or ester thereof is in the range of from about 0.0001 to about 100 mg/kg body weight per day, preferably from about 0.001 to about 50 mg/kg body weight per day.

17. A method to increase the rate and extent of growth of animals, to increase the milk or wool produktion of animals, or for the treatment of ailments, the method comprising administering to a subject in need thereof an effective amount of a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof, or of a composition according to any one of the claims 11, 12 or 14.

18. A compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof for use as a medicament.

19. Use of a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof for the preparation of a medicament for stimulating the release of growth hormone from the pituitary.

20. Use of a compound according to any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof for the preparation of a medicament for

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00266

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/60, C07K 5/02, C07K 7/02, A61K 38/07, A61K 38/08
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS, WPI, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9615148 A2 (GENENTECH, INC.), 23 May 1996 (23.05.96) --	1-14,18-20
P,X	WO 9517423 A1 (NOVO NORDISK A/S), 29 June 1995 (29.06.95) --	1-14,18-20
A	WO 9304081 A1 (ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND), 4 March 1993 (04.03.93) -- --------	1-14,18-20

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 Sept 1996	27 -09- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

administration to animals to increase their rate and extent of growth, to increase their milk or wool production, or for the treatment of ailments.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00266

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 15-17
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [X] Claims Nos.: 1-9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The formulation of claims 1-9 is so complicated because of the long lists of cascading substituents that it does not comply with Article 6 PCT prescribing that claims shall be clear and concise. For this reason the search has been mainly limited to the examples.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/09/96

International application No.
PCT/DK 96/00266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A2- 9615148	23/05/96	AU-A- 4164496	06/06/96
		IL-D- 115994	00/00/00
WO-A1- 9517423	29/06/95	AU-A- 1272495	10/07/95
		HU-D- 9501947	00/00/00
		IL-D- 112112	00/00/00
		NO-D- 962665	00/00/00
		ZA-A- 9410261	23/06/95
WO-A1- 9304081	04/03/93	WO-A- 9220767	26/11/92
		AU-B- 666673	22/02/96
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		BG-A- 98489	28/02/95
		BR-A- 9206398	27/12/94
		CA-A- 2116120	23/02/93
		CN-A- 1073684	30/06/93
		CZ-A- 9400400	16/11/94
		EP-A- 0605484	13/07/94
		FI-A- 940807	21/02/94
		HU-A- 69178	28/08/95
		HU-D- 9400495	00/00/00
		JP-T- 7507039	03/08/95
		NO-A- 940592	14/04/94
		NZ-A- 244034	28/08/95
		SK-A- 20494	05/10/94
		ZA-A- 9206337	22/04/93